CLAIM + DETAILED DESCRIPTION

[Claim(s)]

[Claim 1]Glycosaminoglycan characterized by comprising the following is made to react, Carboxylic acid and this glycosaminoglycan which are characterized for a carbonyl group of this mixed acid anhydride, a hydroxyl group of this glycosaminoglycan, or an amino group by an ester bond or carrying out an amide bond are the manufacturing methods of an ester bond or a glycosaminoglycan derivative which carries out an amide bond.

A mixed acid anhydride produced by making carboxylic acid and ********** low-grade archil phosphinothioyl react.

A hydroxyl group or an amino group.

[Claim 2]A manufacturing method of the glycosaminoglycan derivative according to claim 1 from which a functional group besides carboxyl Motomochi is protected by a protective group, and carboxylic acid removes this protective group after the above-mentioned ester bond reaction or an amide bond reaction.

[Claim 3]Glycosaminoglycan which has a carboxyl group, and ********** low-grade archil phosphinothioyl are made to react, A manufacturing method of a glycosaminoglycan derivative manufacturing a glycosaminoglycan mixed acid anhydride which consists of this glycosaminoglycan and ********** low-grade archil phosphinothioyl.

[Claim 4]A glycosaminoglycan mixed acid anhydride produced by making glycosaminoglycan which has a carboxyl group, and ********** low-grade archil phosphinothioyl react, A manufacturing method of a glycosaminoglycan derivative in which this glycosaminoglycan, the 1st class amine, or secondary amine making the 1st class amine or secondary amine react, and carrying out the amide bond of a carbonyl group of this mixed acid anhydride and the amino group of this amine carries out an amide bond.

[Claim 5]A manufacturing method given in any 1 paragraph of Claims 1-4 characterized by

making it react under existence of a neutralizer in an anhydrous organic solvent.
[Claim 6]The manufacturing method according to claim 1 or 4 making it react in solution containing a water miscible organic solvent.
[Claim 7]It is the spacer compound joint glycosaminoglycan which has the amino group to which the above-mentioned glycosaminoglycan made chondroitin sulfate, hyaluronic acid, or these carboxyl groups carry out the covalent bond of the diamine as a spacer compound, Carboxylic acid Indomethacin, deoxycholic acid, acetysalicylate, Salazosulfapyridine, methotrexate, leucine enkephalin, Glycosaminoglycan which has carboxylic acid, a hydroxyl group, or an amino group manufactured by a procedure according to claim 1 of being leucine, serine, glycine, or glutamine is an ester bond or a glycosaminoglycan derivative which carries out an amide bond.
[Claim 8]The above-mentioned glycosaminoglycan is chondroitin sulfate or the hyaluronic acid, The 1st class amine or secondary amine Bestatin, tranexamic acid, Adriamycin, A glycosaminoglycan derivative in which glycosaminoglycan which has the carboxyl group manufactured by a procedure according to claim 4 of being methotrexate, phenylalanine, or glutamine, the 1st class amine, or secondary amine carries out an amide bond.

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to a glycosaminoglycan derivative and its manufacturing method.
[0002]
[Description of the Prior Art]There is already a thing which has enough application development and which is not carried out in glycosaminoglycan (it may be hereafter called "GAG") like chondroitin sulfate with a thing with the clear usefulness like the hyaluronic acid which is a treating agent of heparin or arthritis which is a vantithrombotic. On the other hand, the peptide, protein, etc. are the compound groups which had a possibility of becoming a useful drug in order to show various outstanding pharmacological actions, but utilization is difficult in many cases because of instability in the living body. Then, improving stability in the living body by embellishing these compounds with a high molecular compound is known, It is thought that the complex (polysaccharide high Brit) with GAG excellent in biocompatibility, amino acid, the peptide, protein, the lipid, other low-molecular organic compounds, etc. fits such a purpose, both usefulness can be increased or a new mechanism can be given.
[0003]The hyaluronic acid exists naturally all over animal tissue, and has living body reabsorption nature, and toxicological and since an immunological action does not exist, itself For example, a medicine, It is used as cosmetics and what combined a drug and bioactive

peptide with this serves as a medicine of the outstanding drug delivery system. It becomes the medical use material which was excellent in biocompatibility by insolubilizing the hyaluronic acid by chemical modification.

[0004][as a chemical modification method of GAG for obtaining the above-mentioned polysaccharide high Brit] the procedure (Eur.J.Biochem., 1, and 46-50 (1967).) of making GAG suspended in dimethylformamide (DMF), making the pyridine a catalyst, and making it react to the oxychloride, since GAG is insoluble to an organic solvent Since Chem.Express, 6 (9), 647-650 (1991), and GAG have a carboxyl group and are water solubility, The method of combining GAG and amines by processing with an activation condensing agent like a water-soluble carbodiimide (WSC) under existence of N-imide hydroxysuccinate (HONSu) is known among solution.

[0005]The sodium salt of heparin is replaced by ammonium salt or tertiary amine salt, After making it meltable to DMF, the dimethylamino pyridine is made into a catalyst and the procedure of making it react to the symmetrical acid anhydride of carboxylic acid is known (Carbohydr.Res., 236,107-119 (1992)). On the other hand, the reaction which condenses amino acid by the mixed acid anhydride method using dimethyl chloride phosphinothioyl (Mpt-Cl) as a chemosynthesis method of the peptide is known (Chem.Lett., 1, p45-48 (1982)). This procedure is the procedure of reacting the hydroxyl group of amino acid by no protecting in peptide synthesis, and reacting also in the solvent of an alcohol system. The manufacturing method of N-acylamino sugar to which amino sugar guided from carboxylic acid and Mpt-Cl, such as acetic acid, benzoic acid, glycolic acid, and glucuronic acid, such as a mixed acid anhydride, glucosamine, or galactosamine, is made to react is known (JP,61-197589,A).

[0006]However, the mixed acid anhydride method which used ********** low-grade archil phosphinothioyl for the chemical modification of the polysaccharide containing glycosaminoglycan was not used.

[0007]

[Problem to be solved by the invention]In the conventional chemical modification method of GAG, severe reaction conditions, such as heating and a prolonged reaction, are required, and were often accompanied by depolymerize and a side reaction. An activation condensing agent and the activated carboxyl group react to water, and decomposes gradually, and also since the procedure of using an activation condensing agent like WSC had the slow reaction and a superfluous reagent was used for it, it was difficult to control an amidation rate. Since the activation condensing agent used superfluously, its decomposition product, and the elimination of acyl urea which carries out subraw especially by acyl transfer were difficult, it often became a problem.

[0008]Since the symmetrical acid anhydride is unstable the procedure of making the ammonium salt or tertiary amine salt of heparin reacting to the symmetrical acid anhydride of

carboxylic acid, in order to decompose during a reaction, there was a problem that it was difficult to introduce carboxylic acid into desired quantity heparin. The purpose of this invention is to provide the GAG derivative for which GAG can be manufactured by the method of performing chemical modification for a short time under a mild condition, and this procedure.
[0009]
[Means for solving problem]This invention persons succeeded in solving the above-mentioned technical problem by the following composition wholeheartedly as a result of research. Namely, this invention is the spacer compound joint glycosaminoglycan characterized by comprising the following with which a spacer compound was combined, . Carboxylic acid is a physiologically active substance which has 1 or two or more carboxyl groups. Glycosaminoglycan which has carboxylic acid, a hydroxyl group, or an amino group manufactured by a procedure of one above-mentioned description An ester bond or a glycosaminoglycan derivative which carries out an amide bond, The above-mentioned glycosaminoglycan is the spacer compound joint glycosaminoglycan which has the amino group which carried out the covalent bond of the diamine to chondroitin sulfate, hyaluronic acid, or these carboxyl groups as a spacer compound, and carboxylic acid 7-2) Indomethacin, Deoxycholic acid, acetysalicylate, salazosulfapyridine, Glycosaminoglycan which has carboxylic acid, a hydroxyl group, or an amino group manufactured by a procedure of 1 which is methotrexate, leucine enkephalin, leucine, serine, glycine, or glutamine An ester bond or a glycosaminoglycan derivative which carries out an amide bond, The above-mentioned glycosaminoglycan 8-1) Chondroitin sulfate, It is the spacer compound joint glycosaminoglycan by which a spacer compound which has a carboxyl group was combined with hyaluronic acid or these, A glycosaminoglycan derivative in which glycosaminoglycan which has the carboxyl group manufactured by a procedure of four above-mentioned description that the 1st class amine or secondary amine is a physiologically active substance which has the 1st class amino group or the 2nd class amino group, the 1st class amine, or secondary amine carries out an amide bond, 8-2) The above-mentioned glycosaminoglycan is chondroitin sulfate or the hyaluronic acid, The 1st class amine or secondary amine Bestatin, tranexamic acid, Adriamycin, A glycosaminoglycan derivative in which glycosaminoglycan which has the carboxyl group manufactured by a procedure of four above-mentioned description which is methotrexate, phenylalanine, or glutamine, the 1st class amine, or secondary amine carries out an amide bond.
1) A mixed acid anhydride produced by making carboxylic acid and ********** low-grade archil phosphinothioyl react.
Glycosaminoglycan which has a hydroxyl group or an amino group is made to react, . A carbonyl group of this mixed acid anhydride, a hydroxyl group of this glycosaminoglycan, or an amino group is characterized by an ester bond or carrying out an amide bond. Carboxylic acid

and this glycosaminoglycan An ester bond or a manufacturing method of a glycosaminoglycan derivative which carries out an amide bond, 2) A functional group besides carboxyl Motomochi is protected for carboxylic acid by a protective group, A manufacturing method of a glycosaminoglycan derivative of one above-mentioned description from which this protective group is removed after the above-mentioned ester bond reaction or an amide bond reaction, 3) Make glycosaminoglycan which has a carboxyl group, and ********** low-grade archil phosphinothioyl react, A manufacturing method of a glycosaminoglycan derivative manufacturing a glycosaminoglycan mixed acid anhydride which consists of this glycosaminoglycan and ********** low-grade archil phosphinothioyl, 4) A glycosaminoglycan mixed acid anhydride produced by making glycosaminoglycan which has a carboxyl group, and ********** low-grade archil phosphinothioyl react.
. It is characterized by making the 1st class amine or secondary amine react, and carrying out the amide bond of a carbonyl group of this mixed acid anhydride, and the amino group of this amine. A manufacturing method of a glycosaminoglycan derivative in which this glycosaminoglycan, the 1st class amine, or secondary amine carries out an amide bond, 5). A manufacturing method given in either of the above 1-4 characterized by making it react under existence of a neutralizer in an anhydrous organic solvent, 6) A manufacturing method the above 1 making it react in solution containing a water miscible organic solvent or given in four and the 7-1 above-mentioned glycosaminoglycan are a hydroxyl group or an amino group to chondroitin sulfate, hyaluronic acid, or these.

[0010]Hereafter, it explains in detail about this invention. Carboxylic acid and ********** low-grade archil phosphinothioyl (it is hereafter described as Rpt-X.) which are used by this invention X is halogen and Rpt is JI low-grade archil phosphinothioyl group:general formula $R_2P=S$ (). [among a formula] R -- the alkyl group of the carbon numbers 1-4 -- a methyl group is shown preferably., [the mixed acid anhydride (henceforth "the mixed acid anhydride A") produced by making react] It is obtained by making this carboxylic acid and 40 ** of Rpt-X react for 10 minutes after for 1 minute preferably from for 1 minute at 0 to 25 ** from -25 ** for 1 hour. [the glycosaminoglycan mixed acid anhydride (henceforth "the mixed acid anhydride B") produced by making the glycosaminoglycan and Rpt-X which have a carboxyl group react] It is obtained by making this GAG and Rpt-X that have a carboxyl group react under the same conditions as the preparation conditions of the above-mentioned mixed acid anhydride A.
[0011]The generation reaction of the mixed acid anhydride A is denoted by the following formulas (1).
R'-COOH+Rpt-X -> R'-COORpt (1)
(R' is a residue except one carboxyl group of carboxylic acid.) The generation reaction of the mixed acid anhydride B is denoted by the following formulas (2).

gag-COOH+Rpt-X -> gag-COORpt (2)
(gag is a residue except one carboxyl group of GAG.)
In this invention, GAG is a concept containing the derivative which introduced the spacer compound etc. into the GAG itself and the GAG itself.
[0012]They are also materials of the new GAG derivative which is a kind of the GAG derivative generated by the procedure of this invention, and is produced by reacting to this, the 1st class amine, or secondary amine further, the mixed acid anhydride B, i.e., gag-COORpt, of the above-mentioned (2) formula.
[0013]The carboxylic acid used for the reaction of the above-mentioned formula (1) may include all the compounds that have a carboxyl group which can react to Rpt-X, and may be the compound from which the functional groups (for example, an amino group, a hydroxyl group, etc.) besides carboxyl Motomochi of this compound were protected by the protective group. Amino acid which specifically protected the amino group as carboxylic acid () [Fmoc-Gln-OH and] Fmoc-Leu-OH, Z-Leu-OH, Boc-Leu-OH, Fmoc-Pro-OH, Boc-Gly-OH, Z-Gly-OH, Fmoc- here Fmoc-Ser(O^{t} Bu)-OH etc. 9-fluorenyl methyloxy carbonyl group, A benzyloxycarbonyl group and Boc- Z- The 3rd butoxycarbonyl group, ^{t}Bu shows a tertiary butyl group, respectively. Peptide which protected the amino group like the case of the above-mentioned amino acid, The physiologically active substance which has a useful pharmacological action with a carboxyl group in intramolecular. for example, indomethacin, deoxycholic acid, and acetysalicylate. Salazosulfapyridine, nicotinic acid, thioctic acid, indole-triacetic acid, This compound that retinoic acid, methotrexate, etc. combined dicarboxylic acid with the compound which has an amino group or a hydroxyl group by a publicly known procedure as a spacer compound, and gave the carboxyl group, Aliphatic carboxylic acid (palmitic acid, linoleic acid, linolenic acid, etc.), cinnamic acid, salicylic acid, trans-epoxy succinic acid, etc. are mentioned.
[0014]As GAG which has a carboxyl group used for the reaction of the above-mentioned formula (2), hyaluronic acid, chondroitin sulfate, chondroitin, dermatan sulfate, heparin, heparan sulfate, etc. are mentioned. Since especially the hyaluronic acid and chondroitin sulfate are excellent in respect of biocompatibility, the activity persistency of a GAG derivative, etc., they are preferred. although above GAG is not limited to the origin and a molecular weight -- general -- the molecular weight about 10,000-5 million -- about 20,000-2 million is mentioned preferably. Although all of separated type or salt type GAG can be used, The sodium salt or potassium salt alkali salt, such as alkali metal salt, alkaline earth metal salt, and amine (for example, tertiary amine, such as triethylamine, N-methyl-morpholine, and diisopropylethylamine etc.) salt, is generally desirable especially apt to obtain is preferred.
[0015]As GAG which has the above-mentioned carboxyl group, it is also compoundable by combining publicly known compounds, such as a spacer compound which has a carboxyl

group, with a publicly known procedure so that it may have a carboxyl group at least. As a spacer compound used for synthesis, the derivative etc. which introduced the protective group into dicarboxylic acid, amino acids, or these carboxyl groups are mentioned.

[0016]As a spacer compound, although R^1-(CH_2) n -R^2 (an R^1=R^2= carboxyl group or the carboxyl group of which R^1= protection was done and an R^2= amino group, and n are 2-18) is mentioned, it is not restricted to this, for example. What is necessary is here, for the protective group just to be able to carry out deprotection of the protective group of a carboxyl group after the GAG creation which combined the spacer compound.

[0017]"GAG which has a carboxyl group" defines this invention as what is not included for convenience at "carboxylic acid." although Rpt-X can use a publicly known thing, a chloride, a bromide, etc. are preferably used for it -- as a low-grade alkyl group -- the alkyl group of the carbon numbers 1-4 -- the thing of a methyl group is used preferably. Specifically, dimethyl chloride phosphinothioyl (Mpt-Cl) is illustrated.

[0018]The base for neutralizing the hydrochloric acid by which it is generated with formation (activation of a carboxyl group) of the mixed acid anhydride A or B in the case of the generation reaction of the mixed acid anhydride A or B when a carboxyl group is free acid, It is preferred to carry out equimolecular amount addition of the tertiary amine, such as triethylamine, N-methyl morpholine, and diisopropylethylamine, with Rpt-X preferably. When a carboxyl group is salt of this base, it is not necessary to add a base further.

[0019]The above-mentioned generation reaction Dimethylformamide (DMF), N,N-dimethylacetamide (DMA), It is carried out in the organic solvent which was single as for dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), chloroform, dichloromethane, etc., or was mixed, and a desirable anhydrous organic solvent. For example, when making carboxylic acid react to Rpt-X and manufacturing the mixed acid anhydride (activated) A, it is made to react in aprotic organic solvents, such as chloroform and DMF. In activating similarly the carboxyl group of GAG which has a carboxyl group and manufacturing the mixed acid anhydride B, It can be made to react using what was solubilized to aprotic organic solvents, such as DMF, in replacing alkali metal salt (sodium salt, potassium salt, etc.) or alkaline earth metal salt of the carboxyl group of GAG, or a sulfuric acid group, etc. by ammonium salt or tertiary amine salt by a publicly known procedure. In this case, since disassembly of a mixed acid anhydride will be suppressed if the moisture which uses and remains an after-dissolution molecular sieve etc. to an organic solvent in GAG is removed, compared with the case where this operation is not performed, the introductory rate of the below-mentioned 1st class amine or secondary amine improves.

[0020]Although it is also possible to use Rpt-X superfluously to GAG which has carboxylic acid or a carboxyl group, if equimolar (1.0 Eq) is used preferably, 1.0-1.2 Eq of desired mixed acid anhydrides can usually be obtained. If said symmetrical acid anhydride method is used in

order for the symmetrical acid anhydride method and the mixed acid anhydride method to be known by the acid anhydride method and to obtain a desired acid anhydride, GAG which has carboxylic acid about equimolar and a carboxyl group in the mixed acid anhydride method of this invention may be sufficient to carboxylic acid of 2 Eq or more being required.

[0021]Thus, as it is, although the mixed acid anhydride obtained can also be isolated, when the mixed acid anhydride A makes GAG which has a hydroxyl group or an amino group, and the mixed acid anhydride B react to the 1st class amine or secondary amine, a glycosaminoglycan derivative is also generable. The reaction of the mixed acid anhydride A and GAG containing a hydroxyl group is denoted, for example by the following formulas (3).

[0022]

R'-COORpt+HO-gag ->R'-COO-gag (3)

The reaction of the mixed acid anhydride A and GAG which has an amino group is denoted by the following formulas (4), for example.

$R'\text{-}COORpt+H_2N\text{-}gag\text{->}R'\text{-}CONH\text{-}gag$ (4)

The reaction with the mixed acid anhydride B, the 1st class amine, or secondary amine is denoted by the following formulas (5), when a reaction with the 1st class amine is illustrated, for example.

[0023]

$gag\text{-}COORpt+H_2N\text{-}A$ ->gag-CONH-A (5)

(A expresses the residue excluding one amino group from amine.)

It can introduce by making the mixed acid anhydride A react to the hydroxyl group of this GAG, and making an ester bond form by the reaction of said formula (3), when introducing carboxylic acid into GAG which has a hydroxyl group. Under the present circumstances, although the solution and the anhydrous organic solvent containing a water miscible organic solvent are mentioned, when the merit of reaction efficiency is taken into consideration, it is preferred [the solvent used for a reaction] that it is an anhydrous organic solvent.

[0024]As a water miscible organic solvent, specifically The dioxane, tetrahydrofuran (THF), N,N-dimethylacetamide (DMA), acetamide, DMF, dimethyl sulfoxide (DMSO), hexamethylphosphoric triamide (HMPA), N-methyl pyrrolidone, pyridine, etc. are mentioned. The solution which includes about 0 to 50% of range as said solution by one or more sorts of totals chosen from either of said illustrated water miscible organic solvents is mentioned.

[0025]The solvent which said illustrated water miscible organic solvent and chloroform, dichloromethane, etc. specifically had as an anhydrous organic solvent, or was mixed is mentioned. [single] As GAG which has a hydroxyl group, GAG(s), such as the hyaluronic acid, chondroitin sulfate, chondroitin, dermatan sulfate, heparin, heparan sulfate, and keratan sulfate, the derivative of those, etc. are mentioned. It may be the compound which combined with these GAG(s) the spacer compound which has a hydroxyl group.

[0026]As the concrete procedure, the mixed acid anhydride A Sodium salt or potassium salt of GAG (separated type) and GAG, etc., Or 60 ** of sodium salt or potassium salt of GAG, etc. is made to react to GAG replaced by ammonium salt or tertiary amine salt from for 5 minutes at 0 to 25 ** preferably from -25 ** for 24 hours. Under the present circumstances, it is preferred to add the catalyst of esterification reactions, such as N and N-dialkyl aminopyridine system catalyst especially 4-dimethylaminopyridine (DMAP), or 4-pyrrolidinopyridine, and the reaction in still milder conditions of it is attained by this catalyst addition.
[0027]It is preferred to add an inorganic base like tertiary amine, such as triethylamine, diisopropylethylamine, pyridine, and N-methyl morpholine, or sodium hydrogen as a neutralizer of acid in order to neutralize the acid generated by a reaction. With the reaction of said formula (4), [the mixed acid anhydride A and GAG which has an amino group] By the reaction of said formula (5), or the mixed acid anhydride B, the 1st class amine, or secondary amine. ("GAG which has an amino group, the 1st class amine, or secondary amine" may be hereafter called amino component collectively) When making it react, -60 ** is 0 ** to 25 ** preferably from 25 **, and it can be made to react from for 5 minutes for 24 hours in the solution containing the inside of 2 desirable hours after for 30 minutes and the above-mentioned anhydrous organic solvent, or a water miscible organic solvent. Under the present circumstances, it is preferred to add the above-mentioned neutralizer as a neutralizer of acid in order to neutralize the acid (JI low-grade alkylthio phosphinic acid) generated with advance of a reaction. 0-80 volume % is mentioned as moisture content of this solution.
[0028]The compound which changed into the amino group the substituted amino groups (an acetylamino group, a sulfoamino group, etc.) of the amino sugar of GAG illustrated as GAG which has the aforementioned hydroxyl group as GAG which has an amino group used for this invention, for example, a deacetylation derivative etc., is mentioned. What is considered as the spacer compound combination GAG which carries out the covalent bond of the spacer compound which has an amino group, for example, the diamine, the amino acid, etc. to the hydroxyl group or carboxyl group of GAG, and has an amino group, Or it can be referred to as GAG which has an amino group also by introducing into the reducing terminal of GAG the spacer compound which has an amino group, and considering it as the spacer compound combination GAG. If GAG which combined with the reducing terminal of GAG the spacer compound which has an amino group is used, it will become possible to combine carboxylic acid with the reducing terminal of GAG, i.e., one specific place.
[0029]After carrying out the ester bond of carboxyl groups, such as amino acid, and the hydroxyl group of GAG which protected the amino group as a spacer compound, for example as a method of introducing a spacer compound, The procedure of carrying out deprotection, the procedure of carrying out the amide bond of one amino group of the diamine, and the carboxyl group of GAG, And the method of mixing the reducing terminal and diamine of GAG

in the suitable solvent, making it join together as a Schiff base, and combining GAG and the diamine according to reduction amination by processing with a reductant further preferably, etc. are mentioned.

[0030]As amino acid which protected the amino group as a spacer compound, natural amino acids with arbitrary glycine, leucine, etc. can be used. omega-amino acid of arbitrary carbon numbers like 6-aminocaproic acid, etc. can also be used, and the degrees of freedom as a spacer can be increased in this case. The 3rd butoxycarbonyl (Boc) group etc. which are removed as a protective group of an amino group with removable 9-fluorenyl methyloxy carbonyl (Fmoc) group and a trifluoro acetic acid solution by for example, the benzyloxycarbonyl (Z) group removable at catalytic reduction and a base are preferred.

[0031]When using the diamine as a spacer compound, the lysine which is also the diamine (for example, 1,6-hexanediamine etc.) and amino acid which are shown by H_2N-$(CH_2)_n$-NH_2 (n is 2-18 preferably) etc., its ester body, etc. can be used. It is good also as the spacer compound combination GAG which carries out an ester bond to the carboxyl group of GAG, using the amino alcohol which protected the amino group as a spacer compound, ranks second, and has an amino group by carrying out deprotection.

[0032]As a method of introducing a spacer compound, it excels in the amide bond, or the simplicity and the cost target of operation of a well known method, such as carrying out an ester bond, at the carboxyl group of GAG using carbodiimides. The carboxyl group of above GAG may be activated by Rpt-X, it may be considered as the mixed acid anhydride B, and this may be made to react to the diamine by the reaction of said formula (5).

[0033][as the 1st class amine or secondary amine used when it is activated as the mixed acid anhydride B and makes the carboxyl group of GAG react to the 1st class amine or secondary amine] For example, amino acids (glutamine, phenylalanine, etc.), amino acid ester (phenylalanine benzyl ester etc.), the peptide, peptide ester, peptide amide, protein, and the physiologically active substance (tranexamic acid.) that has a useful pharmacological action with an amino group in intramolecular Cycloserin, Bestatin, amino cephalosporin acid, the pyridoxamine, Lipid (phosphatidylethanolamine etc.) etc. which have the compound and amino sugar which combined amino acid or the diamine as a spacer compound, and gave the amino group, and amino groups (glucosamine, galactosamine, etc.), such as Adriamycin and methotrexate, are mentioned.

[0034]It is possible to refine by publicly known procedures, such as ethanol precipitation and ******, after a reaction with a reaction with GAG which has the above-mentioned mixed acid anhydride A, a hydroxyl group, or an amino group, the mixed acid anhydride B and the 1st class amine, or secondary amine. When the product deposits after a reaction, a ** collection can be carried out with a glass filter etc., it can depend when that suitable solvent, such as bicarbonate-of-soda water, water, and ethanol, washes one by one, and an object can be

obtained by simple refining operation.

[0035]Said formula (3), [in the reaction of - (5)] [the quantity of the mixed acid anhydride to GAG which has a hydroxyl group, or an amino component, and a catalyst] Although it may be suitably selected by the number of request introduction of carboxylic acid, the 1st class amine, or secondary amine to the kind and GAG of the kind of desired GAG derivative, i.e., the kind of GAG, a molecular weight, or a mixed acid anhydride, etc., What is necessary is for what is necessary to be just to use the mixed acid anhydride of the 1.0 to 3.0 time mole of the number of request introduction, in making an amino component and an amide bond form generally, and just to use the mixed acid anhydride of the 2.0 to 10 time mole of the number of request introduction, in making the hydroxyl group and ester bond of GAG form. The introductory rate of carboxylic acid to a GAG derivative, the 1st class amine, or secondary amine can be defined as a percentage of the number of introductory moles per GAG composition disaccharide unit. Measurement of the integrated intensity of proton NMR can determine the number of introductory moles.

[0036][what according to the manufacturing method of this invention you choose the amino acid which protected the amino group as carboxylic acid, and is made to react by the procedure of said formula (4) as the mixed acid anhydride A] [by removing this amino protecting group after combining the carboxyl group of the amino acid which protected the amino group by the procedure of this invention to the amino group of GAG which has an amino group, and repeating the operation which combines N-protection amino acid by the procedure of this invention again] It is also possible to perform peptide synthesis which expands amino acid one by one on GAG, and to compound a GAG-peptide-bond object. In this case, 9-fluorenyl methyloxy carbonyl (Fmoc) group promptly removable in for example, 10% diethylamine solution etc. as a protective group of an amino group etc. are preferred.

[0037]

[Working example]A work example explains this invention in detail below.

Sodium chondroitin sulfate (10g) of the introductory average molecular weight 30,000 of the 1,6-hexanediamine as a spacer to example of preparation 1 chondroitin sulfate was dissolved in water (100 ml), and DMF (100 ml) was added. After adding the DMF solution (30 ml) of N-hydroxy benzotriazol (HOBt) (40 millimole), and 1,6-hexanediamine and 2 hydrochloride (20 millimole), the DMF solution (40 ml) of dicyclohexylcarbodiimide (DCC) (20 millimole) was added at room temperature. Irrigation elimination of the dicyclohexyl urea which deposited was carried out on the glass filter by ethanol after 1 evening churning under room temperature. After ethanol irrigation, after refining the precipitation obtained on the glass filter with an ethanol sedimentation method further 3 times, when reduced pressure drying was carried out, the chondroitin sulfate in which 1,6-hexanediamine was introduced was used as white powder, and was obtained 7.67g. The result of measurement of 270-MHz proton NMR, The

introductory rate of the above-mentioned diamine per composition disaccharide unit of chondroitin sulfate was 32% from the intensity ratio (3:2.60) of the signal of the acetyl group origin of chondroitin sulfate, and the signal (delta= 1.3 - 1.7 ppm) of four methylene origin of (delta= 2 ppm) and a spacer.

[0038]Here, an introductory rate is denoted by the following formulas.

Sodium chondroitin sulfate (3g) of the introductory average molecular weight 30,000 of the lysine methyl ester as a spacer to example of number of introductory moles x100 preparation 2 chondroitin sulfate of the diamine per introductory rate (%) = disaccharide unit was dissolved in water (30 ml), and DMF (30 ml) was added. After adding the lysine (Z) methyl ester hydrochloride (three millimole) and HOBt (six millimole), the DMF solution (10 ml) of DCC (six millimole) was added under ice-cooling. It flowed into sodium-acetate saturation ethanol after 1 evening churning at room temperature, and the produced sedimentation was centrifuged. The obtained sedimentation was dissolved after refining and in water (10 ml) by the ethanol precipitation method further 3 times, and it dialyzed for three days in deionized water. With a 0.22-micron filter, after filtration, when it freeze-dried, the mark compound was used as white powder and obtained 3.08g. The result of measurement of 270-MHz proton NMR, The introductory rate of the lysine (Z) methyl ester per disaccharide unit of chondroitin sulfate was 25% from the intensity ratio (3:1.23) of the signal of the acetyl group origin of chondroitin sulfate, and the signal (delta= 7.3 - 7.5 ppm) of the benzene-ring origin of (delta= 2 ppm) and Z radical. When this thing was given to the catalytic reduction by ammonium formate and 10% palladium carbon in solution and Z radical was removed, lysine methyl ester joint chondroitin sulfate was obtained.

[0039]In the DMF solution (1 ml) of synthetic deoxycholic acid (0.1 millimole) of work-example 1 deoxycholic-acid modification chondroitin sulfate. [the DMF solution (0.2 ml) of triethylamine (0.2 millimole) and dimethyl chloride phosphinothioyl (Mpt-Cl) (0.1 millimole)] It added under room temperature. This thing was added to the water-DMF (1:1, v/v) solution (2 ml) of lysine methyl ester joint chondroitin sulfate (50 mg) of 25% of the modification rate compounded in the example 2 of preparation under ice-cooling 5 minutes afterward. It flowed into sodium-acetate saturation ethanol after 1 evening churning under room temperature, and the produced sedimentation was centrifuged. The obtained sedimentation was dissolved after refining and in water (10 ml) by the ethanol precipitation method further 3 times, and it dialyzed for two days in deionized water. With a 0.22-micron filter, after filtration, when it freeze-dried, the mark compound was used as white powder and obtained 58 mg. The result of measurement of 270-MHz proton NMR, The introductory rate of deoxycholic acid per composition disaccharide unit of chondroitin sulfate was 27% from the intensity ratio (3:0.81) of the signal of the acetyl group origin of chondroitin sulfate, and the signal (delta= 1.3 - 0.7 ppm) of the methyl group origin of the method of one of (delta= 2 ppm) and deoxycholic acid.

[0040]Here, an introductory rate is denoted by the following formulas.
Lysine methyl ester joint chondroitin sulfate (50 mg) of 25% of the modification rate compounded in the example 2 of synthetic preparation of number of introductory moles x100 work-example 2 acetysalicylate modification chondroitin sulfate of deoxycholic acid per introductory rate (%) = disaccharide unit is used, It replaced with deoxycholic acid of the work example 1, and same operation was performed using acetysalicylate (aspirin). However, when making a mixed acid anhydride, acetysalicylate, triethylamine, Mpt-Cl, and those solvent used 5 times the amount of the work example 1. The yield of 56 mg. The introductory rate of aspirin (acetysalicylate) was 28%.
[0041]The DMF solution (0.5 ml) of triethylamine (0.5 millimole) and Mpt-Cl (0.5 millimole) was added to the DMF solution (2 ml) of synthetic Z-leucine (0.5 millimole) of the chondroitin sulfate which combined work-example 3Z-amino acid under room temperature. In addition, triethylamine (0.5 millimole) was further added under ice-cooling of this thing in the DMF solution (2 ml) of triethylamine salt (100 mg) and 4-dimethylaminopyridine (DMAP) of after 5 minutes and chondroitin sulfate (0.5 millimole). Sodium hydrogen solution (5 ml) was added 5% after 1 evening churning under room temperature, it flowed into sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. The obtained sedimentation was dissolved after refining and in water (10 ml) by the ethanol precipitation method further 3 times, and it dialyzed for three days in deionized water. With a 0.22-micron filter, after filtration, when it freeze-dried, the mark compound was used as white powder and obtained 107 mg. The introductory rate of Z-leucine per composition disaccharide unit of chondroitin sulfate was 38% from the intensity ratio (3:1.9) of the signal of the acetyl group origin of chondroitin sulfate, and the signal of the benzene-ring origin of (delta= 2 ppm) and Z-leucine as a result of measurement of 270-MHz proton NMR.
[0042]The DMF solution (0.1 ml) of triethylamine (0.1 millimole) and Mpt-Cl (0.1 millimole) was added to the DMF solution (1 ml) of joint AFmoc-Ser($O^{t}Bu$)-OH (0.1 millimole) of the protection amino acid to the reducing terminal of work-example 4 hyaluronic acid under ice-cooling. It adds to the water-DMF (1:1) solution (8 ml) of the hyaluronic acid (100 mg) of the average molecular weight 40,000 which combined 1,6-hexanediamine after 5 minutes and with a reducing terminal with the reducing amination method using sodium cyanoborohydride according this thing to a conventional method under ice-cooling, Furthermore, triethylamine (0.1 millimole) was added. Sodium hydrogen solution (5 ml) was added 5% after 1 evening churning under room temperature, it flowed into sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. The obtained sedimentation was dissolved after refining and in water (10 ml) by the ethanol precipitation method further 3 times, and it dialyzed for seven days in deionized water. With a 0.22-micron filter, after filtration, it freeze-dried and 106 mg was obtained as white powder. 56 units of hyaluronic acid per serine monad were

combined per disaccharide from the intensity ratio (3:0.162) of the signal of the acetyl group origin of the hyaluronic acid, and the signal of (delta= 2 ppm) and tertiary butyl (^{t}Bu) radical origin as a result of measurement of 270-MHz proton NMR.

[0043]B) The place which replaced with the hyaluronic acid of the joint work example 4A of the protection amino acid to the reducing terminal of chondroitin sulfate, and carried out the same reaction using chondroitin sulfate of the average molecular weight 30,000, 98 mg of objects were able to be obtained from the chondroitin sulfate (100 mg) which 1,6-hexanediamine combined. 60 units of chondroitin sulfate per serine monad was combined per disaccharide from NMR.

[0044]The sodium salt of the hyaluronic acid of the synthetic average molecular weight 40,000 of the work-example 5 salazosulfapyridine modification hyaluronic acid was made into triethylamine salt with the conventional method using ion exchange resin. The mixed acid anhydride of salazosulfapyridine (0.2 millimole) prepared by the same procedure as the work example 1 was added to what added DMAP (0.2 millimole) to the DMF solution (15 ml) of this hyaluronic-acid triethylamine salt (200 mg), and bottom of room temperature 1 evening churning was carried out. After the same post-processing as the work example 3, the mark compound was used as yellow powder and obtained 117 mg. The introductory rate of the salazosulfapyridine for which it asked from NMR was 5%.

[0045]Under ice-cooling, DMF (2 ml) of triethylamine (one millimole) and Mpt-Cl (one millimole) was added to the DMF (2 ml) solution of synthetic indomethacin (one millimole) of work-example 6 indomethacin modification chondroitin sulfate (it introduces by an amide bond). Chondroitin sulfate of the average molecular weight 30,000 in which 1 and 6-hexanediamine combined this thing as a spacer 5 minutes afterward (200 mg) They are preparation and an introductory rate with the same procedure as the example 1 of preparation. In addition to 32% of water-DMF (1:1) solution (8 ml), triethylamine (one millimole) was added continuously. When [which was the same as that after 1 evening churning and of the work example 3 at room temperature] processed, the mark compound was used as white powder and obtained 242 mg. The introductory rate for which it asked from NMR was 24%.

[0046]The DMF solution (1 ml) of triethylamine (one millimole) and Mpt-Cl (one millimole) was added to the DMF solution (1 ml) of synthetic indomethacin (one millimole) of the work-example 7 indomethacin modification hyaluronic acid (it introduces by an ester bond) under ice-cooling. This thing is added to the mixed solution of 50 ml of the triethylamine salt (100 mg) / DMF solution of the hyaluronic acid of the average molecular weight 800,000, and 4-dimethylaminopyridine (DMAP) (0.5 millimole) / DMF solution (1 ml) under ice-cooling in 5 minutes, Furthermore, triethylamine (one millimole) was added. Sodium hydrogen solution (2 ml) was added 5% after 1 evening churning under room temperature, it flowed into sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. Further 5 times,

80%, by ethanol, it washed and refined and reduced pressure drying of the obtained sedimentation was carried out, and the mark compound was used as white powder and obtained 140 mg.

[0047]The indomethacin introduction rate computed from comparison with the indomethacin content calculated from the calibration curve beforehand computed with absorptiometry and the hyaluronic-acid content calculated from the carbazole-sulfuric-acid method was 29.8%. In accordance with the procedure of related work-example 7 description of a work-example 8 indomethacin charge and an introductory rate, the charge (reaction equivalent) of indomethacin and the relation of the introductory rate were investigated. The hyaluronic acid also used the thing of 50,000, 300,000, and 800,000 for the average molecular weight.

[0048]The result was indicated to drawing 1. It turns out that the relation between the amount of preparations and an introductory rate will be the first [about] relation if drawing 1 is seen. When seen with the average molecular weight of the hyaluronic acid, 50,000 and 300,000 showed the almost same action, but about the thing of 800,000, it turns out that reactivity is falling on the whole. This is considered that it is the influence by the increase in the viscosity by the amount of polymers.

[0049]After dissolving 80 mg (0.2 millimole as a disaccharide unit) of hyaluronate sodium of the manufacture average molecular weight 1 million of the 9t of work-examples-butoxy carbo NIRUGU ricin modification hyaluronic acid in 20 ml of water, 10 ml of dioxane was added. A carboxyl group by Mpt-Cl in this solution. [as a mixed acid anhydride] After adding 3 ml of activated dimethylformamide solutions of 175 mg (1.0 millimole) of t-butoxy carbo NIRUGU ricin, triethylamine 139microl (1.0 millimole), and 122 mg (1.0 millimole) of 4-dimethylaminopyridine under ice-cooling, 1-hour and half churning was continued at room temperature. The obtained solution was filled with 150 ml of sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. By carrying out reduced pressure drying of the obtained sedimentation after refining by an ethanol precipitation method further 3 times, the mark t-butoxy carbo NIRUGU ricin modification hyaluronic acid was used as white powder, and was obtained 75 mg. The introductory rate per hyaluronic-acid composition disaccharide unit of the t-butoxy carbo NIRUGU ricin for which it asked from the intensity ratio of the signal of the hyaluronic-acid acetyl group origin of NMR and the signal (delta= 1.5 ppm) of (delta= 2 ppm) and tertiary butyl group origin was 11%.

[0050]Replace with the manufacture t-butoxy carbo NIRUGU ricin of the work-example 10 benzyloxycarbonyl glycine modification hyaluronic acid and the glycine modification hyaluronic acid, and. [209 mg (1.0 millimole) of benzyloxycarbonyl glycine (it is described also as Z glycine)] It used and the same procedure as the work example 7 was followed.

[0051]After refining, by carrying out reduced pressure drying, the mark benzyloxycarbonyl glycine modification hyaluronic acid was used as white powder, and was obtained 74 mg. The

signal of the hyaluronic-acid acetyl group origin of NMR. The introductory rate per hyaluronic-acid composition disaccharide unit of the benzyloxycarbonyl glycine for which it asked from the intensity ratio of the signal (delta= 7.5 ppm) of the benzyl origin of (delta= 2 ppm) and the benzyloxycarbonyl glycine was 11%.

[0052]60 mg (0.15 mmol/unit) of benzyloxycarbonyl glycine modification hyaluronic acid is dissolved in 50 ml of water, After 15 mg of palladium (Pd) activated charcoal and 19 mg (0.3mmol) of ammonium formate agitating at room temperature in addition for 6 hours 10% under argon atmosphere, tales doses of Pd activated charcoal and ammonium formate were added and agitated again. 6 hours afterward, after carrying out same operation again, a 0.22-micrometer filter removed activated charcoal, and after dialyzing solution for two days in deionized water, a 51-mg white thing was obtained by lyophilization. Elimination of the peak (delta= 7.5 ppm) of benzyl origin was checked by NMR.

[0053]After dissolving 80 mg (0.2 millimole as a disaccharide unit) of hyaluronate sodium of the manufacture average molecular weight 150,000 of the 11t of work-examples-butoxy carbo NIRUGU ricin modification hyaluronic acid (molecular weight 150,000) in 20 ml of water, 10 ml of dioxane was added. The carboxyl group of t-butoxy carbo NIRUGU ricin by Mpt-Cl in this solution. [as a mixed acid anhydride] After adding the activated dimethylformamide solution of 175 mg (1.0 millimole) of t-butoxy carbo NIRUGU ricin, triethylamine 139microl (1.0 millimole), and 122 mg (1.0 millimole) of 4-dimethylaminopyridine under ice-cooling, 1-hour and half churning was continued at room temperature. The obtained solution was filled with 150 ml of sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. By carrying out reduced pressure drying of the obtained sedimentation after refining by an ethanol precipitation method further 3 times, the mark t-butoxy carbo NIRUGU ricin modification hyaluronic acid was used as white powder, and was obtained 55 mg. The introductory rate per hyaluronic-acid composition disaccharide unit of the t-butoxy carbo NIRUGU ricin for which it asked from NMR was 5%.

[0054]The sodium salt of chondroitin sulfate of the synthetic average molecular weight 30,000 of a work-example 12A chondroitin sulfate mixed acid anhydride and Bestatin modification chondroitin sulfate was made into triethylamine salt with the conventional method using ion exchange resin. The DMF solution (0.2 ml) of Mpt-Cl (0.1 millimole) was added to the DMF solution (2 ml) of this chondroitin sulfate triethylamine salt (50 mg) under ice-cooling, and the chondroitin sulfate mixed acid anhydride was prepared.

[0055]after 5 minutes, Bestatin (0.05 millimole), and triethylamine (0.05 millimole) -- water (0.5 ml) was added further and bottom of room temperature 1 evening churning was carried out. After adding sodium hydrogen solution (5 ml) 5%, it flowed into sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. The obtained sedimentation was dissolved after refining and in water (10 ml) by the ethanol precipitation method further 3 times,

and it dialyzed for four days in deionized water. With a 0.22-micron filter, after filtration, when it freeze-dried, the mark compound was used as white powder and obtained 57 mg. The introductory rate of Bestatin per disaccharide unit of chondroitin sulfate was 8% from the intensity ratio (3:0.41) of the signal of chondroitin sulfate acetyl group origin, and the signal of the benzene-ring origin of (delta= 2 ppm) and Bestatin as a result of measurement of 270-MHz proton NMR.

[0056]B) When it changed to Bestatin of the synthetic work example 10A of tranexamic acid modification chondroitin sulfate and same operation was performed using tranexamic acid, the mark compound was obtained at 15% of the introductory rate.

The sodium salt of chondroitin sulfate of the synthetic average molecular weight 30,000 of work-example 13 phenylalanine benzyl ester modification chondroitin sulfate was dissolved in DMF as triethylamine salt with the conventional method using ion exchange resin, the molecular sieve of 4A was added, and this solution was dried. The DMF solution (0.4 ml) of Mpt-Cl (0.2 millimole) was added to the DMF solution (2 ml) of this chondroitin sulfate triethylamine salt (100 mg) under ice-cooling. 5 minutes afterward, tosyl salt (0.2 millimole) and triethylamine (0.4 millimole) of phenylalanine benzyl ester were added, and bottom of room temperature 1 evening churning was carried out. After adding sodium hydrogen solution (5 ml) 5%, it flowed into sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. The obtained sedimentation was dissolved in water (10 ml) after refining by the ethanol precipitation method further 3 times, and it dialyzed for three days in deionized water. With a 0.22-micron filter, after filtration, when it freeze-dried, the mark compound was used as white powder and obtained 103 mg. The result of measurement of 270-MHz proton NMR, The introductory rate of the phenylalanine benzyl ester per disaccharide unit of chondroitin sulfate was 25% from the intensity ratio (3:2.5) of the signal of the acetyl group origin of chondroitin sulfate, and the signal of the benzene-ring origin of phenylalanine benzyl ester with (delta= 2 ppm).

[0057]An introductory rate was 13% when this DMF solution by a molecular sieve was not dried in the same experiment as the above.

Sodium salt of chondroitin sulfate of the synthetic average molecular weight 30,000 of work-example 14 Adriamycin modification chondroitin sulfate was made into tetrabutylammonium salt in accordance with a conventional method using ion exchange resin. DMF (0.1 ml) and triethylamine (0.1 millimole) of Mpt-Cl (0.05 millimole) were added to DMF (10 ml) solution of this chondroitin sulfate tetrabutylammonium salt (50 mg) under ice-cooling. Solution (1 ml) of Adriamycin (10 mg) after 10 minutes was added, and one evening was agitated at room temperature. When processed in accordance with a conventional method, a mark compound was used as red powder and obtained 50 mg. An introductory rate for which it asked from NMR was 9%.

[0058]Adriamycin was not able to be introduced into chondroitin sulfate depending on publicly known carbodiimide method.
DMF solution (1 ml) of triethylamine (0.25 millimole) and Mpt-Cl (0.25 millimole) was added to DMF solution (1 ml) of synthetic methotrexate (0.25 millimole) of work-example 15 methotrexate modification hyaluronic acid (it introduces by an ester bond) under ice-cooling. This thing is added to a mixed solution of 50 ml of the triethylamine salt (100 mg) / DMF solution of hyaluronic acid of the average molecular weight 800,000, and 4-dimethylaminopyridine (DMAP) (0.125 millimole) / DMF solution (1 ml) under ice-cooling in 5 minutes, Furthermore, triethylamine (0.25 millimole) was added. Sodium hydrogen solution (2 ml) was added 5% after 1 evening churning under room temperature, it flowed into sodium-acetate saturation ethanol, and produced sedimentation was centrifuged. Ethanol washed obtained sedimentation 80% further 3 times. After dissolving obtained precipitation in water, in deionized water, one evening was dialyzed and it freeze-dried, and a mark compound was used as white powder and obtained 77 mg.
[0059]The methotrexate introduction rate computed from comparison with the methotrexate content calculated from the calibration curve beforehand computed with absorptiometry and the hyaluronic-acid content calculated from the carbazole-sulfuric-acid method was 2.7%.
The sodium salt of the hyaluronic acid of the synthetic average molecular weight 800,000 of a work-example 16 hyaluronic-acid mixed acid anhydride and the methotrexate modification hyaluronic acid (it introduces by an amide bond) was made into triethylamine salt with the conventional method using ion exchange resin. The DMF solution (0.25 ml) of Mpt-Cl (0.25 millimole) was added to 50 ml of DMF solution of this hyaluronic-acid triethylamine salt (100 mg) under ice-cooling, and the hyaluronic-acid mixed acid anhydride was prepared.
[0060]5 minutes afterward, 1 ml of DMF solution and triethylamine (0.25 millimole) of methotrexate (0.25 millimole) were added, and bottom of room temperature 1 evening churning was carried out. After adding sodium hydrogen solution (5 ml) 5%, it flowed into sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. The obtained sedimentation was dissolved after irrigation and in water (10 ml) by ethanol 80% further 3 times, and it dialyzed for three days in deionized water. When it freeze-dried, the mark compound was used as white powder and obtained 83 mg.
[0061]The methotrexate introduction rate computed from comparison with the methotrexate content calculated from the calibration curve beforehand computed with absorptiometry and the hyaluronic-acid content calculated from the carbazole-sulfuric-acid method was 3.0%.
The DMF solution (1 ml) of triethylamine (one millimole) and Mpt-Cl (one millimole) was added to the DMF solution (1 ml) of synthetic Fmoc-Gln-OH (one millimole) of the work-example 17 glutamine modification hyaluronic acid (it introduces by an ester bond) under ice-cooling. This thing is added to the mixed solution of 50 ml of the triethylamine salt (100 mg) / DMF solution

of the hyaluronic acid of the average molecular weight 800,000, and 4-dimethylaminopyridine (DMAP) (0.5 millimole) / DMF solution (1 ml) under ice-cooling in 5 minutes, Furthermore, triethylamine (one millimole) was added. Sodium hydrogen solution (2 ml) was added 5% after 5-hour churning under room temperature, it flowed into sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. The obtained sedimentation was dissolved after irrigation and in water (10 ml) by ethanol 80% further 3 times, and it dialyzed in deionized water on the 1st. When it freeze-dried, the Fmoc-Gln-hyaluronic acid was used as white powder and obtained 127 mg.

[0062]The glutamine introduction rate computed from comparison with the Fmoc-Gln-OH content calculated from the calibration curve beforehand computed with absorptiometry and the hyaluronic-acid content calculated from the carbazole-sulfuric-acid method was 31.3%. Diethylamine solution (20 ml) was further added to 57 mg of this Fmoc-Gln-hyaluronic acid 15% under ice-cooling, and it agitated at room temperature for 1 hour. Sodium hydrogen solution (2 ml) was added 5%, it flowed into sodium-acetate saturation ethanol, and the produced sedimentation was centrifuged. The obtained sedimentation was dissolved after irrigation and in water (10 ml) by ethanol 80%, and it dialyzed in deionized water on the 1st. When it freeze-dried, the mark compound was used as white powder and obtained 39 mg.

[0063]The extension Fmoc-leucine (0.5 millimole) of the peptide on work-example 18 chondroitin sulfate was made to react to Mpt-Cl by the same procedure as the work example 3, and it was considered as the mixed acid anhydride. The water-DMF (1:1) solution (4 ml) of the hexanediamine joint chondroitin sulfate (the average molecular weight 30,000, 32% of a spacer introduction rate, 100 mg) compounded in the example 1 of preparation was dropped at this thing, and triethylamine (0.5 millimole) was added further. When post-processing was carried out like the work example 3, Fmoc-Leu-hexanediamine chondroitin sulfate was used as white powder, and was obtained 99 mg. The introductory rate of the leucine for which it asked from 270-MHz proton NMR was 18% (it is 100% to a spacer) to the carboxyl group of chondroitin sulfate. Diethylamine solution (5 ml) was added to Fmoc-Leu-hexanediamine chondroitin sulfate (95 mg) 10% under ice-cooling, and it was neglected for 1 hour. When it filtered and freeze-dried with a 0.22-micron filter after dialysis for two days by deionized water, Leu-hexanediamine chondroitin sulfate was used as white powder, and was obtained 85 mg. As a result of measurement of 270-MHz proton NMR, the signal of leucine origin remained and the signal of Fmoc radical origin had disappeared.

[0064]Like the following, combination of the Fmoc-phenylalanine, the Fmoc-glycine, the Fmoc-glycine, and the Fmoc-tyrosine and elimination of the Fmoc radical were repeated, and, finally leucine enkephalin modification chondroitin sulfate was obtained.

[0065]

[Effect of the Invention]As explained above, this invention is useful as a new manufacturing

method of a GAG derivative promising as a material of DDS or a medical material. Since there being few side reactions, such as depolymerize of GAG, and its reaction time are short since the manufacturing method of this invention is a reaction under mild conditions, and the mixed acid anhydride is comparatively more stable still, control of a modification rate is the procedure excellent in the comparatively easy point. What is also made to react in a hydrous organic solvent (it acylates) is made.

[0066]The effect which excelled [derivative / which physiologically active substances manufactured by the procedure of this invention, such as indomethacin, Adriamycin, and salazosulfapyridine, combined / GAG] in fields, such as the durability of water solubility and activity, compared with the physiologically active substance independent case is expected.

[Translation done.]